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Research Article

Effects of cholecystokinin-octapeptide and cerulein on ovine digestive motility under cholinergic blockade

Krzysztof W. Romański

Department of Biostructure and Animal Physiology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland; E-mail: krzysztof.romanski@up.wroc.pl

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ABSTRACT

In sheep, contribution of cholinergic system to the control of gastrointestinal motility by cholecystokinin is unknown. Accordingly, in six non-fasted rams chronic experiments were conducted and the myoelectrical activity of abomasal antrum, duodenum and jejunum was recorded before and after injection of atropine (two doses), pirenzepine (two doses), hexamethonium or atropine plus hexamethonium followed or not by injection of three doses of cholecystokinin octapeptide or cerulein. In the course of the experiments performed, the anticholinergic drugs and hormones suppressed spike burst activity both in abomasal antrum and small bowel and inhibited the migrating myoelectric complex and 'minute rhythm'. When the hormones were injected after cholinergic blockade, they induced longer inhibitory effects than cholinergic blockade alone. In the small bowel, some stimulatory effects were observed as well. The higher dose of pirenzepine and remaining anticholinergics induced rebound excitation in the small bowel, but when followed by cholecystokinin peptide administration, no rebound effect was denoted. Hexamethonium given alone or in combination with atropine followed by cholecystokinin peptide caused stronger inhibitory effect than that of atropine or pirenzepine. It is concluded that cooperation exists between the cholinergic system and cholecystokinin in the control of gastrointestinal motility in sheep and the role of nicotinic mechanisms is greater than that of muscarinic mechanisms.

Keywords: Ram; Abomasal antrum; Small intestine; Electromyography; Cholecystokinin octapeptide; Cerulein; Anticholinergic drug.

1. INTRODUCTION

Cholecystokinin (CCK) represents the meaningful peptide hormone and neuromodulator produced by endocrine cells in the gastrointestinal mucosa and by neurons in both central and peripheral nervous system [1, 2]. The hormone modulates motor function both in the stomach and small bowel and the character of motility alterations mostly depends upon the animal species and gastrointestinal segment [3]. CCK, as gastrin, its closely related peptide, can inhibit the abomasal motility and gastric emptying in the ruminants [4, 5]. It was reported that in sheep, CCK inhibits the arrival of the migrating motor complex (MMC) and accelerates small intestinal transit [6, 7]. Cerulein, the amphibian CCK, depresses abomasal motility, stimulates small intestinal contractions and disrupts the MMC in this species [8-13]. Both these peptides seem to be able to modulate also the 'minute rhythm' (MR) in the ovine small bowel [12, 14].

Most of these effects are similar to those observed in monogastric species [15-17]. There is also the increasing evidence that the nervous system strongly contributes to the action of CCK upon the gastrointestinal motility and that the action of the hormone is largely neuronal, both central and peripheral [2, 18]. When CCK was injected intracerebroventricularly, it disrupted the MMC pattern in the dog and rat [19, 20]. Thus, the mechanism of CCK action on gastrointestinal motility is composed. In sheep, CCK evoked central effect on forestomach motility suggesting that in this species CCK can indirectly modulate the gastrointestinal motor function [21]. It has also been reported that the vagus nerve participates in the control of gastrointestinal motility by CCK and the central effects are thus possible to occur [6, 22-24]. Peripheral administration of CCK does not seem to exert central effect directly since CCK probably cannot cross the blood-brain barrier [25]. This does not exclude the possibility of the involvement of peripheral neurons in CCK action upon the gastrointestinal motility. The cholinergic system could be the first candidate for such cooperation. It is well known, also in sheep, that the cholinergic system controls effciently the gastrointestinal motility and the cholinergic blockade can inhibit contractions and disrupt both the MMC and MR [26-28]. Several reports indicate that peripheral cholinergic system interferes in the actions of CCK upon the gastrointestinal motility while the problem has not yet been satisfactorily explored [29-31]. However, nothing is known about these mechanisms in sheep. Thus, the aim of this work was to demonstrate the modulatory role of cholinergic mechanisms in the action of CCK octapeptide (CCK-OP) and cerulein upon the antral, duodenal and jejunal motility in conscious rams. It is hypothesized that obtained results can embrace the question how does CCK cooperate with the cholinergic system in the control of ovine gastrointestinal motility.

2. MATERIALS AND METHODS

2.1. Animal preparation

Six healthy, adult, non-fasted rams, each weighing 38-44 kg, were used in the chronic experiments performed in the study. Animals were kept in cages with normal light rhythm. Before and after surgery, they were habituated for the experiments. Under general and local anaesthesia, right lateral laparotomy was performed and five platinum bipolar electrodes and one strain gauge force transducer (RP Products, Madison) were sewn onto the gastrointestinal serosa of each ram. The electrode localization was as follows: 1 - the abomasal antrum, 4 cm before the pyloric ring, 2 - the duodenal bulb, 6 cm below the pyloric ring, 3 - the duodenum, 56 cm distally to the pyloric ring, 4 - the first jejunal electrode, 256 cm distally to the pyloric ring, 5 - the second jejunal electrode, 356 cm distally from the pyloric ring.

The strain gauge force transducers, calibrated individually, were attached onto the duodenal serosa nearby the third electrode in four of these rams. Marked electrode and transducer wires were exteriorized over the skin, soldered to the plug in the designed order and fixed onto the integument. Within 2-3 days following the surgery, animals gradually returned to normal feeding and then the fodder (good quality hay and the grain mixture) was not restricted, except in the course of the experiment. The drinking water was restricted only during the experiment. The postsurgical recovery period lasted at least 10 days and thereafter the skin sutures were removed. Other details of the experimental model applied in this study were reported elsewhere [13, 32].

2.2. Experimental design

The total of 252 randomized experiments, each lasting 5-8 h, were performed. While the experiment was performed in one ram, the second ram was also present in the experimental room for company. Just before motility recording, the silastic cathether was introduced into the left jugular vein of each ram for intravenous drug and hormone administration. The myoelectric and motor activity was recorded throughout the experiments using the multichannel electroencephalograph (Reega, Alvar Electronic, Paris), also adapted for mechanical recordings. Before the experiments, the efficacy of the cholinergic blockade was checked in three rams with the use of bethanechol preceding atropine or pirenzepine administration and DMPP preceding

hexamethonium administration. During the first part of the experiment (i.e. before drug and hormone administration), the gastrointestinal electromyography and motility recordings were conducted. The normal motility patterns, namely the MMC and the MR were identified. All the MMC phases, including phase 2a and 2b, were regularly identified during this initial control period according to the appropriate criteria [10, 33-35]. 5 ml of 0.15 M NaCl was slowly administered intravenously during early phase 2b of the MMC. During this part of the experiment, at least one full MMC cycle was recorded. In the course of the second part of the experiment, drugs were given intravenously during phase 2b of the next MMC cycle at the doses tested previously. The various doses of cholecystokinin octapeptide (CCK-OP, Sincalide, Squibb Inst., Princeton) and cerulein (Takus, Farmitalia Carlo Erba, Milan) were injected after cholinergic blockade. Following drug administration, each lasting 30 s, the myoelectrical recordings were continued until the normal motility was restored, especially till the arrival of the normal, non-ectopic phase 3 of the MMC. The reference experiments (first series) with cholinergic blockade applied alone were conducted during which the following anticholinergic drugs were injected: atropine sulfate (At, Sigma, St. Louis) at the doses 0.02 and 0.1 mg/kg, each dose given in separate experiment, (2) pirenzepine dihydrochloride (Pi, Sigma, St. Louis) 0.02 and 0.1 mg/kg, each dose given in separate experiment, (3) hexamethonium bromide (Hx, Sigma, St. Louis) 2.0 mg/kg, (4) At 0.1 in combination with Hx 2.0 mg/kg given also in separate experiments. In the course of the proper experiments (second series), one of two CCK peptides was administered following cholinergic blockade. Each dose of CCK-OP (20, 200 or 2000 ng/kg) and cerulein (1, 10 or 100 ng/kg) was preceded by administration of the same anticholinergic drug and dose during separate experiments. The time lag between the smaller doses of Pi or At administration and CCK peptide administration was not longer that one min. In the case of the remaining types of cholinergic blockade, CCK peptides were given 1-2 min after the anticholinergic drug. At least two days overpassed between two consecutive experiments while after the experiments with Hx, duration of the break lasted at least three days.

2.3. Analysis of data

All the recordings were visually analysed in order to identify the motility patterns and to evaluate the intensity and arrangement of the spike bursts and contractions. During the initial part of the experiments, i.e. before cholinergic blockade, the correctness of motility recordings, mainly the occurrence of the normal motility patterns, was confirmed. In the abomasal antrum, duration of spike burst inhibition was calculated not only when complete lack of the spike bursts was present, but also comprised the periods in which the inhibition reached at least 70% of the maximal spike burst amplitude. In the small bowel, duration of the spike burst inhibition (regardless of the arrival of stimulatory events, i.e. the phase 3 of the MMC, premature phase 3, MR and rebound excitation) was calculated following the anticholinergic drug administration (results treated as the reference values) and following CCK peptide administration always preceded by cholinergic blockade. Duration of MMC disruption was measured from the end of anticholinergic drug administration till the arrival of the first phase 3 of the MMC at the given recording channel (the reference value). The time lags from the end of CCK peptide administration (after cholinergic blockade) until the onset of the first phase 3 of the MMC were measured as well. Finally, duration of MR inhibition, from the end of the anticholinergic drug administration till the arrival of the first MR episode in the given recording channel and the time lag from the end of CCK peptide injection (administered after cholinergic blockade) till the arrival of the first MR episode were measured. After stimulatory effects, evoked during the inhibitory period, the spike burst inhibition was still present for some time in almost all cases. These periods were also taken into account during calculations. After termination of the whole inhibitory period, the normal gastrointestinal motility reappeared.

2.4. Statistical elaboration of data

All the data were collected, analysed and grouped, and the mean values with standard deviations (±S.D.) were calculated. All the data were rounded and presented as the whole numbers.

The normality of data distribution was checked and the appropriate comparisons were performed using the variance analysis followed by the Student *t*-test for paired values [36].

2.5. Ethical approval

Protocol of study and informed consent were in compliance with the Helsinki convention and were approved by Local Ethics Committee.

3. RESULTS

During control parts of the experiments, saline injections did not evoke any effect upon the gastrointestinal motility.

Among the anticholinergic substances, Hx was the strongest inhibitory drug as to the antral myoelectrical activity although the spike burst inhibition was complete only in two of six experiments and lasted 2-3 min. Partial inhibition (less than 70% of the maximal spike burst amplitude) was approximately 2-3 times longer in this region than the periods of complete inhibition. Similar situation was observed following the combined cholinergic blockade, i.e. At plus Hx (At+Hx) administration (Table 1). After the smallest dose of CCK-OP administration preceded by Hx or by At + Hx, duration of the inhibitiory periods was slightly but significantly shorter than that after the relevant anticholinergic drug dose given alone. When the highest doses of CCK-OP and cerulein were applied after cholinergic blockade, the inhibitory periods were significantly longer as compared with the higher doses of At or Pi and with Hx or At + Hx administration (Figure 1). Cerulein induced more pronounced effect than CCK-OP (Table 1). Following the higher dose of At, cerulein exerted dose-dependent inhibitory effect upon the antral spike burst amplitude. The inhibitory effect evoked by the maximal doses of both CCK peptides, given after Hx, lasted longer than in response to Hx applied alone. After Pi and At, the effect of CCK peptide was slightly shorter than that after Hx (Table 1).

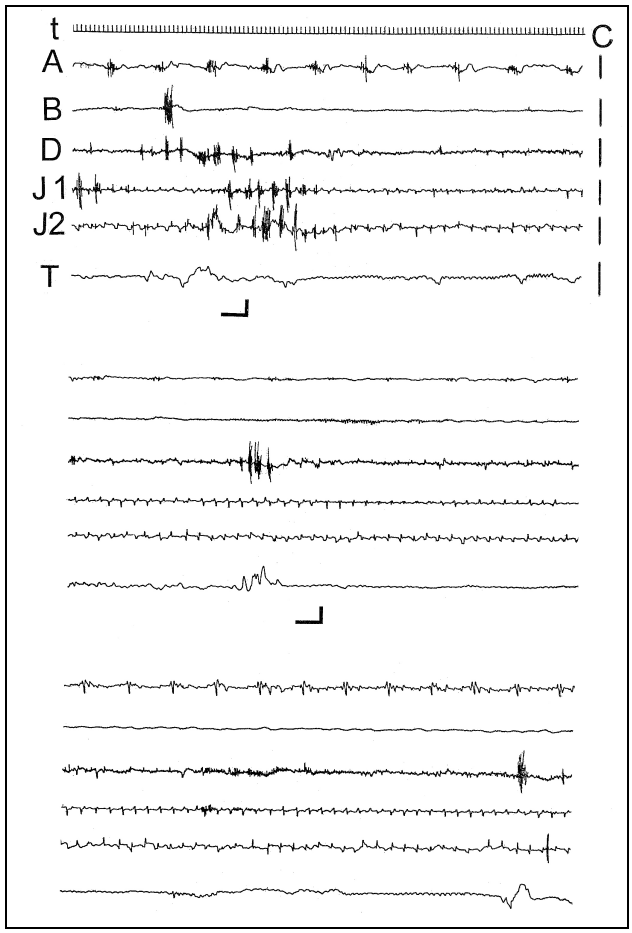

Figure 1. The effects of muscarinic blockade followed by cerulein administration upon the myoelectrical and motor activity of ovine abomasal antrum, duodenum and jejunum. Upper panel: administration of atropine at the dose 0.1 mg/kg (marked). Middle panel: continued recording, administration of cerulein at the dose 100 ng/kg (marked). Lower panel: next two minutes following cerulein administration. Note partial inhibition of the antral spike bursts after atropine and complete inhibition after cerulein administration. Complete inhibition of the intestinal motility in response to cholinergic blockade followed by cerulein administration with lack of the rebound effect, except the presence of single spike burst in the duodenum resembling the residual 'minute rhythm' during cerulein administration is also visible.

Explanations: t - time in seconds; A - electromyographical recording from the abomasal antrum; B - the duodenal bulb, D - the duodenum; J1 - proximal jejunum; J2 - recording from the the second jejunal electrode; T - mechanical recording from the duodenal strain gauge force transducer; C - electrode and transducer calibration, 100 μV and 5g, respectively; ┘(the bent bar) - termination of drug or hormone administration. Other explanations are as in the chapter Materials and Methods.

Table 1. Duration of the spike burst inhibition in the abomasal antrum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Atropine		Pirenzepine		Hexam. 2.0	Atropine 0.1 + Hexam. 2.0
		0.02	0.1	0.02	0.1		
No CCK peptide	Mean	0	1.0	0	0	4	5
	±S.D.	0	0.0	0	0	2	2
CCK-OP 20.0	Mean	0	0^{c}	0	0	1^{a}	2^{a}
	±S.D.	0	0	0	0	0	1
CCK+OP 200.0	Mean	0	0^{c}	0	0	3^{y}	4
	±S.D.	0	0	0	0	1	2
CCK+OP 2000.0	Mean	2^{cz}	2^{az}	4^{cz}	6^{cz}	8^{az}	9^{y}
	±S.D.	1	1	1	3	2	4
Cerulein 1.0	Mean	0	0^{c}	0	1^{b}	2	4^{x}
	±S.D.	0	0	0	0	1	1
Cerulein 10.0	Mean	0	1^{c}	0	0	3	5
	±S.D.	0	0	0	0	1	2
Cerulein 100.0	Mean	0	5^{cz}	4^{cz}	7^{cz}	11^{cz}	10^{x}
	±S.D.	0	2	1	3	5	4

Explanations: doses of the anticholinergic drugs expressed in mg/kg, doses of CCK peptides expressed in ng/kg. Statistical significances: n=6; $^{a}P<0.05$, $^{b}P<0.01$, ^{c} $P<0.001$ vs. reference value (no CCK peptide administration); $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$ vs. the relevant value obtained in response to the lowest dose of CCK peptide. Other explanations as in the chapter Material and methods.

Table 2. Partial excitatory events observed during inhibitory periods evoked by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

	Duodenal bulb						Duodenum						Jejunum 1						Jejunum 2					
	At		Pi		Hx	At+ Hx	At		Pi		Hx	At+ Hx	At		Pi		Hx	At+ Hx	At		Pi		Hx	At+ Hx
	l	h	l	h			l	h	l	h			l	h	l	h			l	h	l	h		
No CCK	0	0	0	0	4	3	1	3	2	2	6	3	2	4	4	5	1	2	0	2	4	4	0	2
OP 20.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0
OP 200.0	1	0	0	0	0	0	3	0	3	0	1	1	0	1	5	1	1	0	0	0	5	1	0	0
OP 2000.0	5	1	0	1	0	0	4	1	4	6	0	2	2	3	1	1	0	1	0	0	0	4	0	2
Cer 1.0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0
Cer 10.0	2	0	1	0	0	0	3	2	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0
Cer 100.0	6	0	3	3	0	0	5	4	5	4	0	1	4	0	5	6	3	1	3	0	0	5	0	0

Values represent numbers of the experiments in which the excitatory event arrived. The excitatory events comprised three types of episodes. The premature phase 3 was observed in response to Pi administration at the lower dose. The rebound excitation was seen in the experiments with remaining types of the cholinergic blockade and after the lower dose of Pi followed by CCK peptide administration. The presence of single spike bursts was denoted once after the moderate dose of CCK peptide or often 2-3 times following its highest dose. l. - lower dose (0.02 mg/kg), h. higher dose (0.1 mg/kg); OP - cholecystokinin octateptide; Cer - cerulein. Other explanations as in Table 1.

In the small intestine, unlike in antrum, administration of the anticholinergic drugs followed or not by CCK peptides, induced various stimulatory effects that arrived during the inhibitory periods. The premature phase 3 was evoked in the most cases only by Pi given alone at the lower dose (as shown in Table 2). Administration of At, the higher dose of Pi, Hx and At + Hx, not followed by CCK peptide, evoked clear rebound excitation exhibiting stationary character. When the animals were treated by the lower dose of Pi and then by CCK peptide, no premature phase 3 arrived and instead, the rebound excitation was observed, but not in all the animals studied (Figure 2). When CCK peptide followed the administration of At, the higher dose of Pi, Hx and At + Hx, no rebound excitation was observed although the spike burst inhibition was incomplete (Figure 3). Following the cholinergic blockade, the arrival of usually one or two separate stronger spike bursts was often observed in the duodenum during or just after CCK peptide injection at the moderate or high dose (Table 2, Figure 1). These single spike bursts resembled the MR-forming spike bursts. Sometimes, following the moderate dose of the peptide, more than one isolated spike burst was observed. These effects are also presented in Table 2.

Duration of the spike burst inhibition was different in the various small intestinal segments. When the cholinergic blockade was applied, the spike burst inhibition (calculated including periods when the excitatory effects occurred during the inhibitory response, namely the premature phase 3, rebound excitation or the isolated spike burst) lasted longer in the duodeno-jejunum than in the duodenal bulb (Table 3A, B). Among the anticholinergic drugs, Hx exerted the strongest inhibitory effect, especially in the jejunum, where the Hx-evoked rebound excitation was usually absent (Figure 4). At induced rebound excitation mostly in the duodeno-jejunum and rather not in the duodenal bulb. When CCK peptides were given after cholinergic blockade, they often exerted significant, dose-related effect. Following the highest dose of both CCK peptides, the inhibitory period lasted much longer than after both lower doses. The effect of cerulein was often more pronounced that the relevant effect of CCK-OP. It was seen mostly in the jejunum. Introduction of the lower dose of At followed by cerulein, inhibited the spike bursts for the period longer than in the experiments in which the same dose of cerulein injection was preceded by the higher dose of At (Table 3A, B). Similar observation concerned also Pi. In all the regions examined, administration of Hx or At + Hx combined with both CCK peptides evoked significantly longer inhibitory effects than those of At and Pi when injected before CCK peptide, regardless of their doses (Table 3A,B, see also Figure 5). Cerulein, given at the lowest dose and preceded by the both doses of Pi, produced significantly shorter inhibitory response in the duodenum than Pi given alone. CCK-OP, used at the lowest dose and preceded by the lower dose of Pi, Hx or by higher dose of At, inhibited spike burst activity in the jejunum for significantly shorter time than the relevant anticholinergic drug given alone (Table 3A, B).

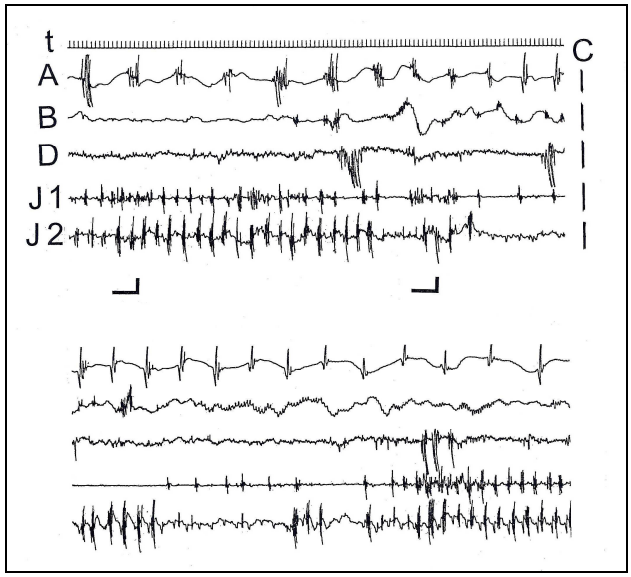

Figure 2. The effects of muscarinic blockade followed by cholecystokinin octapeptide (CCK-OP) administration upon the myoelectrical activity of ovine abomasal antrum, duodenum and jejunum after muscarinic blockade. Upper panel: administration of pirenzepine (Pi) at the dose 0.02 mg/kg (left bar) followed by CCK-OP at the dose 200 ng/kg (right bar). Lower panel: continued recording after OP-CCK administration. Note the stronger inhibitory effect on antral spike burst after OP-CCK than after Pi. Pi did not inhibit the jejunal myoelectric activity and OP-CCK inhibited it in part. Symbol explanations as in Figure 1.

Table 3A. Duration of the spike burst inhibition in the duodenal bulb and the duodenum in response to the cholinergic blockade applied alone and to the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Duodenal bulb						**Duodenum**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**	**2.0**	
No CCK peptide	Mean	4	3	2	4	7	7	6	10	14	15	11	12
	±S.D.	1	1	1	2	2	3	3	4	6	5	4	6
CCK-OP 20.0	Mean	10^{b}	17^{c}	6^{a}	7	16^{a}	29^{c}	8	19	15	12	9	26^{a}
	±S.D.	2	7	2	3	7	7	4	8	6	4	4	7
CCK OP 200.0	Mean	16^{cx}	24^{c}	16^{cz}	5	33^{cx}	42^{c}	13	25^{a}	24	8	26^{ax}	61^{cz}
	±S.D.	4	9	3	2	11	9	5	10	9	3	11	19
CCK OP 2000.0	Mean	13^{b}	39^{cx}	19^{cz}	18^{cx}	48^{cz}	61^{cz}	12	32^{c}	17	19	34^{cz}	93^{cz}
	±S.D.	6	14	5	7	19	15	6	11	8	5	13	27
Cerulein 1.0	Mean	11^{a}	8^{a}	5^{a}	12^{a}	54^{c}	33^{c}	12	9	2^{c}	5^{b}	32^{c}	28^{b}
	±S.D.	5	3	2	5	16	12	4	3	1	2	11	7
Cerulein 10.0	Mean	19^{c}	11^{b}	17^{cy}	46^{cz}	60^{c}	52^{c}	14^{a}	13	12^{z}	9	35^{c}	46^{cx}
	±S.D.	9	4	7	14	17	11	5	5	5	4	14	10
Cerulein 100.0	Mean	23^{cz}	28^{cz}	21^{cz}	16^{b}	66^{c}	58^{c}	18^{b}	25^{ax}	18^{z}	14^{y}	55^{c}	49^{cx}
	±S.D.	7	12	6	7	20	17	6	11	7	4	19	12

Explanations as in Table 1.

Table 3B. Duration of the spike burst inhibition in the upper and more distal jejunum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Jejunum 1						**Jejunum 2**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**	**2.0**	
No CCK peptide	Mean	8	14	7	9	12	13	3	15	3	6	16	12
	±S.D.	3	7	2	3	5	6	1	7	1	3	6	6
CCK-OP 20.0	Mean	6	12	2^{a}	11	7	24	12^{b}	8	2	5	6^{a}	32^{c}
	±S.D.	1	5	1	4	3	7	4	2	1	2	2	8
CCK-OP 200.0	Mean	9^{x}	20	14^{az}	13	8	38^{c}	17^{c}	19^{z}	15^{cz}	7	14	41^{c}
	±S.D.	2	8	5	5	3	8	6	4	6	2	7	12
CCK OP 2000.0	Mean	18^{ax}	26	23^{cz}	22^{bx}	24^{az}	57^{cz}	14^{a}	25^{z}	23^{cz}	12^{x}	16^{x}	54^{c}
	±S.D.	5	11	9	6	7	18	8	6	8	5	6	16
Cerulein 1.0	Mean	11	12	6	7	53^{c}	21	18^{c}	14	9^{a}	3	30	38^{c}
	±S.D.	5	5	2	2	16	8	6	6	3	1	12	13
Cerulein 10.0	Mean	17^{a}	7	11	5	78^{c}	35^{cx}	25^{c}	6^{ax}	14^{c}	6	45^{c}	52^{c}
	±S.D.	6	2	4	2	24	6	11	2	5	3	16	19
Cerulein 100.0	Mean	24^{bx}	8	18^{bz}	6	96^{c}	49^{cz}	28^{c}	7	36^{cz}	8^{x}	63^{cx}	86^{cz}
	±S.D.	9	3	5	2	31	9	11	3	11	3	21	24

Explanations as in Table 1.

Duration of inhibition of phase 3 of the MMC was often long and dependent upon the intestinal segment examined. In the most distal recording channel (jejunum 2), these periods were usually shorter than in the proximal sites since the first phase 3 of the MMC, which arrived after cholinergic blockade applied alone and also after the combination of anticholinergic drugs with CCK peptides, was ectopic. It was started most often just from this distal region (Table 4A, B). Duration of

phase 3 inhibition was longer after Hx or after At + Hx administration than after At or Pi. Despite of the arrival of premature phase 3 following the lower dose of Pi, no inhibitory effect on the regular phase 3 was denoted and the arrived regular phase 3 of the MMC was not ectopic. The premature phase 3 was often ectopic and abortive. When At or Pi were injected, duration of the subsequent phase 3 inhibitory periods was related to the drug dose. When CCK peptide administration followed the cholinergic blockade, the time lags, measured from CCK administration until the appearance of the regular ectopic phase 3, were significantly longer than after cholinergic blockade alone (Table 4A, B). Following the highest doses of CCK peptides, these periods were relatively very long. In the most experiments, the effect of CCK-OP administration was more pronounced than the effect of relevant dose of cerulein, at least in the duodenum and upper jejunum (Table 4A, B).

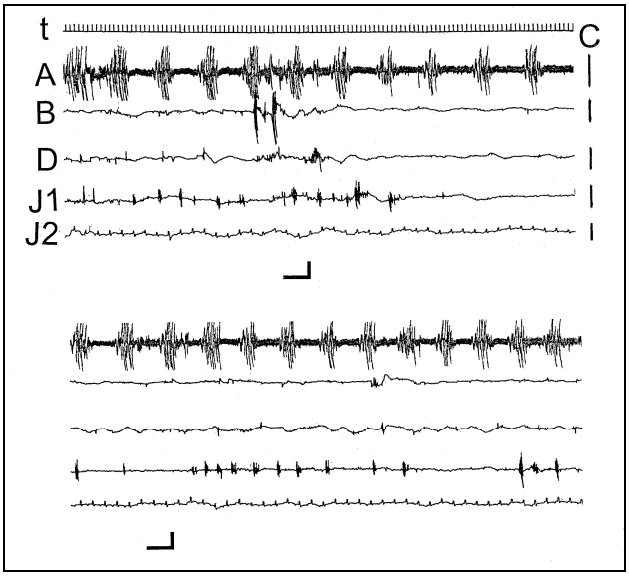

Figure 3. The effects of muscarinic blockade followed by cholecystokinin octapeptide (CCK-OP) administration upon the myoelectrical activity of ovine abomasal antrum, duodenum and jejunum.
Upper panel: administration of pirenzepine (Pi) at the dose 0.1 mg/kg (marked). Lower panel: continued recording and administration of CCK-OP at the dose 200 ng/kg (marked). Note the inhibition of intestinal motility by pirenzepine and lack of rebound effect. CCK-OP exerted slight stimulatory effect in the upper jejunum. No clear inhibition of antral myoelectrical activity is also visible. Symbol explanations as in Figure 1.

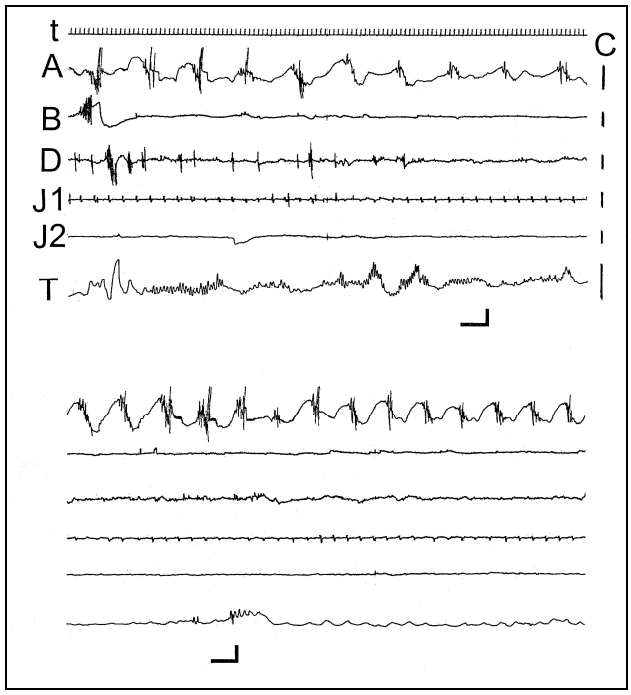

Figure 4. The effects of nicotinic blockade followed by cerulein administration upon the myoelectrical and motor activities in the ovine abomasal antrum, duodenum and jejunum. Upper panel: adninistration of hexamethonium (Hx) at the dose 2.0 mg/kg (marked). Lower panel: continued recording and administration of cerulein at the dose 100 ng/kg (marked). Note the partial inhibition of antral spike bursts by Hx and complete inhibition by cerulein. The electrical and mechanical activity of the small intestine is also inhibited by both of these drugs.
Symbol explanations as in Figure 1.

The time lags between cholinergic blockade and arrival of the first MR episode were usually shorter than phase 3 disruption periods in all the small intestinal segments examined (Tables 4A, B, 5A, B). In the most experiments, duration of MR inhibition was longer following Hx or At + Hx administration than that after At or Pi (Table 5 A, B). Following At injection, this effect was dose-related in all segments examined while after Pi it was rather dose-independent and relatively short. Duration of MR inhibition following CCK-OP and cerulein application after cholinergic blockade exhibited dose-related character, especially in the jejunum. Administration of both CCK peptides, at least at two highest doses, often delayed MR arrival for significantly longer periods than the cholinergic blockade applied alone. These periods were the longest when Hx or At+Hx was followed by CCK peptide administration, especially at its highest dose.

Table 4A. Duration of inhibition of the phase 3 of the migrating myoelectric complex in the duodenal bulb and the duodenum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Duodenal bulb						**Duodenum**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**	**2.0**	
No CCK peptide	Mean	26	62	29	34	73	64	17	63	21	67	56	66
	±S.D.	11	18	8	14	22	19	8	19	9	22	11	17
CCK-OP 20.0	Mean	49[a]	76	45	31	83	54	42[c]	64	46[a]	75	63	52
	±S.D.	10	18	17	10	24	14	6	21	15	24	21	15
CCK-OP 200.0	Mean	68[c]	61	71[c]	117[cz]	131[cy]	87	58[c]	71	61[c]	119	116[c]	89[z]
	±S.D.	21	21	23	38	21	21	20	22	20	39	38	17
CCK-OP 2000.0	Mean	96[cz]	116[c]	74[c]	124[cz]	186[cz]	158[cz]	104[cz]	117[cx]	83[c]	178[cz]	166[cz]	155[cz]
	±S.D.	27	28	14	35	45	39	32	30	37	41	39	37
Cerulein 1.0	Mean	47	56	41	62[a]	78	66	34	58	43	94	81	59
	±S.D.	12	17	18	14	16	17	16	17	16	19	19	16
Cerulein 10.0	Mean	56[c]	70	79[c]	68[c]	148[cz]	121[cy]	65[c]	104[cx]	65[c]	88	146[cz]	120[cz]
	±S.D.	11	21	24	12	35	34	22	29	18	23	34	32
Cerulein 100.0	Mean	87[cz]	98[x]	86[cx]	73[c]	197[cz]	176[cz]	85[cz]	107[cz]	79[c]	74	198[cz]	174[cx]
	±S.D.	20	24	26	19	46	48	18	27	25	21	48	47

Explanations as in Table 1.

Table 4B. Duration of inhibition of the phase 3 of the migrating myoelectric complex in the upper and more distal jejunum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Jejunum 1						**Jejunum 2**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**	**2.0**	
No CCK peptide	Mean	18	38	19	34	45	37	17	37	20	35	35	38
	±S.D.	4	16	7	13	9	14	5	15	5	13	12	16
CCK-OP 20.0	Mean	33[b]	49	48[a]	47	38	53	28	43	27	23	45	37
	±S.D.	8	22	21	13	17	12	11	18	11	8	14	12
CCK-OP 200.0	Mean	57[c]	73	52[c]	120[cz]	62	76[c]	39[a]	54	22	34	59	49
	±S.D.	18	25	16	36	26	19	15	24	7	11	18	11
CCK-OP 2000.0	Mean	102[cz]	86[c]	67[c]	134[cz]	139[cz]	153[cz]	87[cz]	58	66[cz]	67[az]	118[cz]	76[cz]
	±S.D.	30	24	28	35	28	38	21	16	20	17	32	13
Cerulein 1.0	Mean	28	42	54[c]	29	62	60	21	41	18	27	43	31
	±S.D.	12	11	19	12	24	14	8	10	4	11	12	9
Cerulein 10.0	Mean	54[c]	48	58[c]	45	111[c]	122[cz]	39[c]	47	29	39	76[cx]	78[bz]
	±S.D.	16	16	18	21	44	33	11	14	10	17	21	20
Cerulein 100.0	Mean	69[cz]	52	61[c]	48	176[cz]	156[cz]	53[cz]	53	38[az]	54	109[cz]	135[cz]
	±S.D.	19	14	17	19	51	54	17	13	11	21	26	33

Explanations as in Table 1.

These effects were most pronounced in more distal jejunum (Table 5A, B). In the most experiments, initial administration of lower doses of At and Pi potentiated the MR inhibition by CCK peptide even more than pretreatment with their higher doses. When cerulein administration at the

lowest dose was preceded by the higher dose of At, MR inhibition was significantly shortened as compared with the experiments with At alone (Table 5A, B).

Table 5A. Duration of the 'minute rhythm' inhibition in the duodenal bulb and the duodenum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Duodenal bulb						**Duodenum**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**	**2.0**	
No CCK peptide	Mean	14	28	8	7	41	38	8	23	9	11	45	46
	±S.D.	5	7	3	3	10	11	3	10	4	5	11	12
CCK-OP 20.0	Mean	15	31	15	9	52	44	11	32	16	12	45	38
	±S.D.	4	9	5	3	18	13	4	10	5	4	21	11
CCK-OP 200.0	Mean	39^{bz}	48	17^{a}	12	97^{cz}	46	28^{cz}	46	18	13	32	64
	±S.D.	16	19	6	4	18	17	8	17	7	4	8	18
CCK+OP 2000.0	Mean	58^{cz}	79^{cz}	33^{cy}	22^{ax}	146^{cz}	68^{a}	42^{cz}	80^{cz}	26^{b}	22^{ax}	96^{cx}	97^{cz}
	±S.D.	18	22	9	10	38	17	14	23	9	6	31	24
Cerulein 1.0	Mean	18	8^{c}	9	6	56	52	24^{c}	8^{a}	17^{a}	14	36	49
	±S.D.	7	2	3	2	12	16	4	3	4	6	9	17
Cerulein 10.0	Mean	48^{cz}	12^{c}	16	15^{ay}	63	76^{b}	18^{a}	12	26^{c}	18	32	56
	±S.D.	12	4	6	4	19	21	6	5	7	8	12	18
Cerulein 100.0	Mean	10	22^{y}	7	24^{cz}	68^{a}	64	9^{z}	23^{y}	11^{x}	25^{a}	57	55
	±S.D.	4	10	3	7	16	19	2	8	3	8	18	21

Explanations as in Table 1.

Table 5B. Duration of the 'minute rhythm' inhibition in the upper and more distal jejunum by the cholinergic blockade applied alone and by the cholinergic blockade followed by cholecystokinin peptide administration in rams.

		Jejunum 1						**Jejunum 2**					
		Atropine		**Pirenzep.**		**Hx**	**At 0.1 + Hx 2.0**	**Atropine**		**Pirenzep.**		**Hx 2.0**	**At 0.1 + Hx 2.0**
		0.02	**0.1**	**0.02**	**0.1**	**2.0**		**0.02**	**0.1**	**0.02**	**0.1**		
No CCK peptide	Mean	9	19	16	10	42	45	13	23	14	16	54	36
	±S.D.	3	6	5	4	14	12	5	11	7	7	10	11
CCK-OP 20.0	Mean	10	31	16	12	33	26	34^{c}	54^{a}	24	17	44	43
	±S.D.	3	10	6	3	11	7	6	16	9	6	18	11
CCK-OP 200.0	Mean	29^{cz}	47^{b}	19	24^{cz}	48	42	29^{b}	66^{c}	29	30^{ax}	66	66
	±S.D.	11	18	6	4	18	14	8	20	12	7	15	21
CCK-OP 2000.0	Mean	43^{cz}	80^{cz}	25	46^{cz}	96^{cz}	62^{z}	47^{c}	84^{c}	26	45^{cz}	105^{ax}	106^{cz}
	±S.D.	15	21	8	12	24	17	16	25	8	13	41	32
Cerulein 1.0	Mean	33^{c}	9^{a}	11	9	34	28	38^{c}	10	16	22	54	52
	±S.D.	12	3	3	3	9	9	10	3	5	8	17	12
Cerulein 10.0	Mean	30^{c}	13	25^{z}	18^{x}	61^{x}	61^{z}	27^{a}	15	36^{a}	39^{b}	84^{a}	87^{cx}
	±S.D.	11	5	6	6	19	21	9	5	14	13	21	22
Cerulein 100.0	Mean	26^{c}	24^{y}	46^{cz}	25^{ay}	86^{cz}	99^{cz}	31^{a}	24^{y}	42^{by}	38^{b}	119^{cz}	106^{cz}
	±S.D.	8	8	14	9	14	28	11	7	18	12	26	33

Explanations as in Table 1.

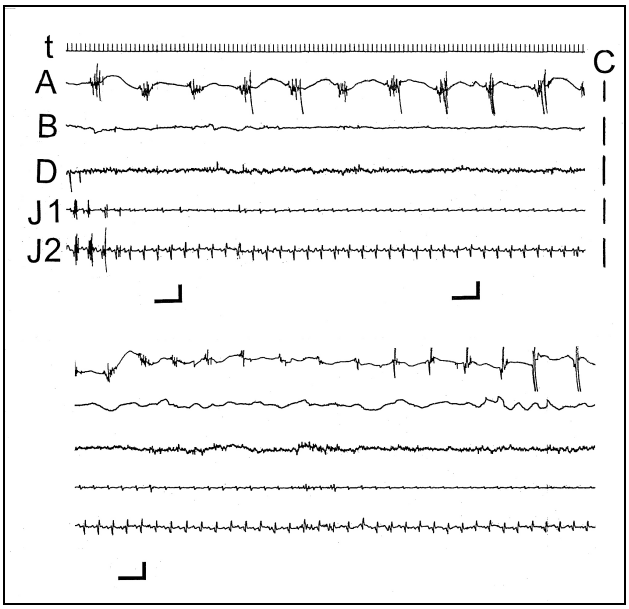

Figure 5. The effects of combined muscarinic-nicotinic blockade followed by cholecystokinin octapeptide (CCK-OP) administration upon the myoelectrical activity of the ovine abomasal antrum, duodenum and jejunum.
Upper panel: adminstration of atropine at the dose 0.1 mg/kg (left bar) and hexamethonium at the dose 2.0 mg/kg (right bar). Lower panel: administration of CCK-OP at the dose 2000 ng/kg (marked). Note the partial inhibition of antral spike burst by anticholinergic drugs and complete inhibition by CCK-OP. The inhibition of the intestinal myoelectrical activity is seen both after cholinergic blockade and CCK-OP administration.
Symbol explanations as in Figure 1.

4. DISCUSSION

The results indicate that CCK profoundly contributes to the control of motility of the ovine abomasal antrum and upper small bowel, and its effects can be efficiently mediated by the cholinergic system. In the abomasal antrum, inhibitory effects were evoked primarily by cholinergic blockade. In sheep, the influence of At and other anticholinergic drugs on the antral spike bursts and contractions is limited as it was observed in the present and previous study [35]. Similar observations were reported in man and dog [30, 37]. Wong and McLeay [38], in the *in vitro* study on ovine antral smooth muscle preparations, did not observe any influences of At or Hx upon the spontaneous contractions. As it was found in the present study, when the anticholinergic drug administration was followed by CCK injection, inhibition of antral spike bursts was much longer than after cholinergic blockade applied without subsequent CCK administration. These effects were also more distinct than the effects of both CCK peptides administered without cholinergic blockade although they were also inhibitory [9, 39]. Thus it is clear that in ovine abomasal antrum, CCK exerts inhibitory effect what was observed also by others [7]. Antral response to CCK is not the same in sheep and dog in which it can be stimulatory [29, 40]. Other studies confirmed further the presence of marked species differences. When CCK was given intraarterially *in vivo* or during *in vitro* studies with the canine antral muscle, it also exerted stimulatory effect [37, 41]. In man, the reported effects of CCK on antral motility are controversial. Its stimulatory effect *in vitro* was confirmed *in vivo* by the inhibitory action of loxiglumide, the CCK receptor antagonist, although the suppressive action of CCK on human antral motility was observed as well [30, 42, 43]. In rats, stimulatory, inhibitory or the lack of the effect was denoted [44, 45]. In the guinea pig, stimulatory action of CCK seems to predominate although the presence of dual effect was also described [46-48]. The effect of CCK on antral motility is, thus, distinct in sheep what suggests that the mechanism of CCK action might be somehow different from that observed in other species. Moreover, the obtained results show that in sheep CCK amplified inhibitory effect evoked by the cholinergic blockade. This effect of CCK was dose-dependent, at least in part, and it also seems to be additive to the effect induced by cholinergic blockade. The existence of cooperation of CCK with acetylcholine has been described [1], but it seems improbable during the efficient cholinergic blockade. This cooperation may concern rather stimulatory than inhibitory action of CCK. The effect of CCK on the ovine gastrointestinal motility can be dual [13, 49], thus it is possible that in the present study the anticholinergic drugs hampered exclusively the stimulatory component of CCK action prolonging the inhibitory effect. At least three pathways of CCK action on antral motility under cholinergic blockade can be considered, however. CCK might be able to evoke the inhibitory effect rather independently of the cholinergic system and this effect could be local and direct on the smooth muscle that represents first possibility.

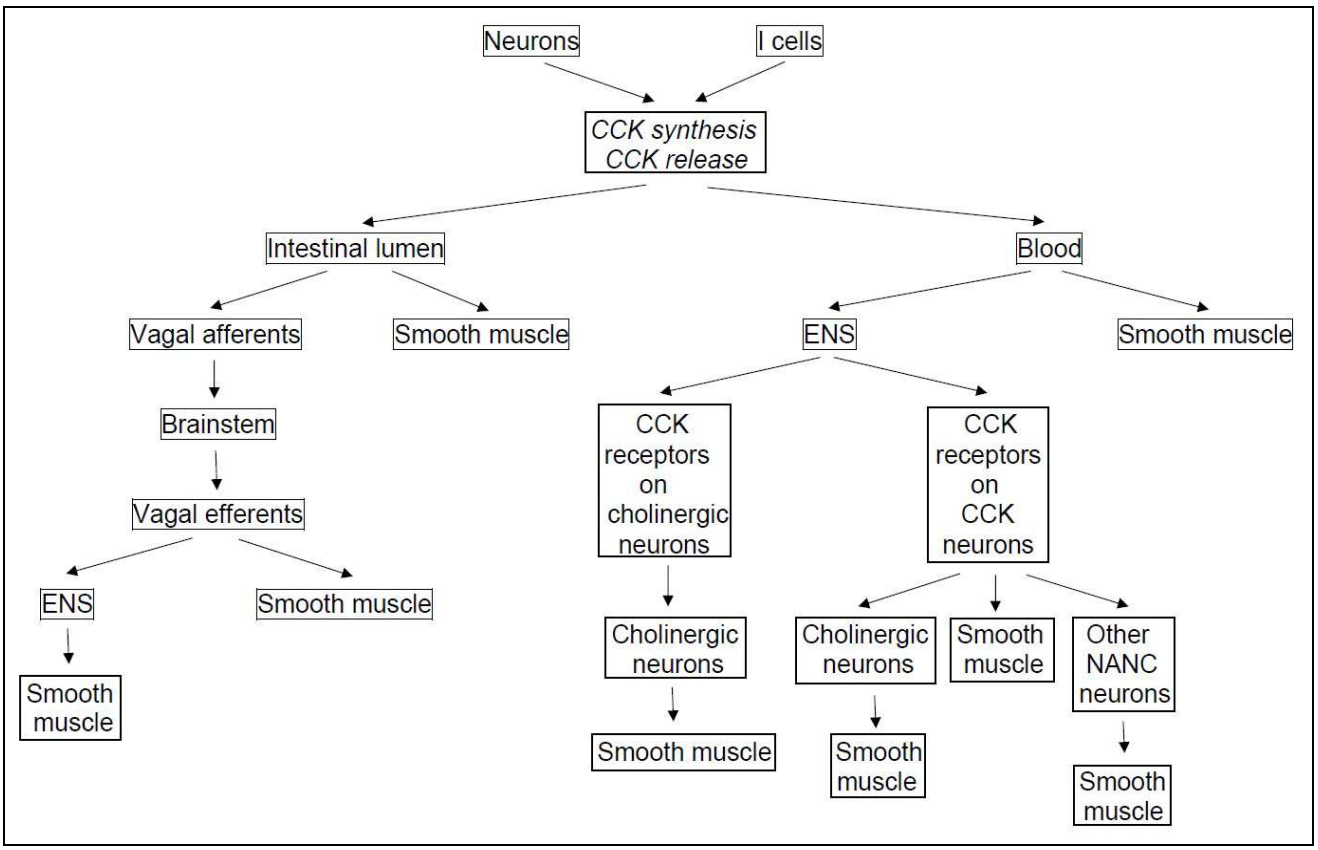

Figure 6. Proposed mechanisms of CCK actions on gastrointestinal motility. Other explanations see text.

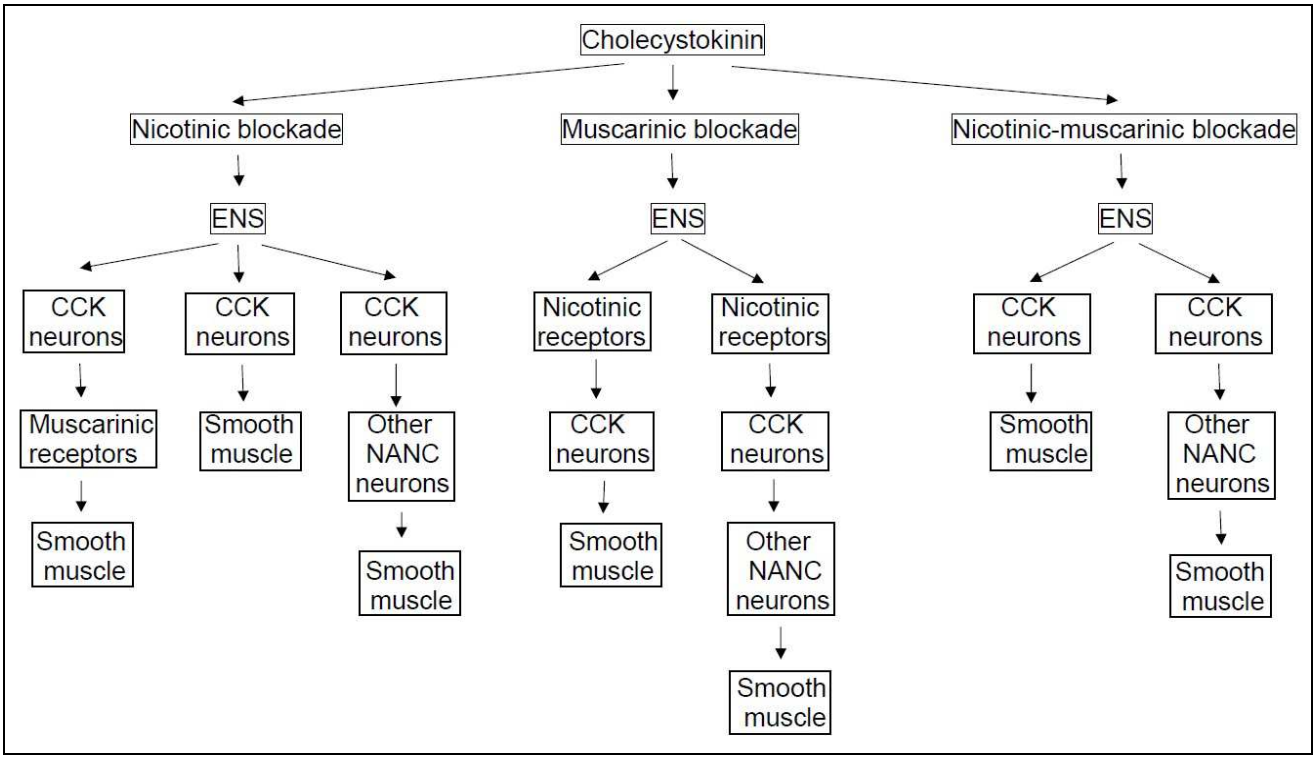

Figure 7. Proposed neuronal CCK actions on gastrointestinal motility during various types of cholinergic blockade. Other explanations see the text.

CCK can also act as a neuromodulator what represents second possibility [28, 50]. Third possibility occurs when the action of CCK could be amplified by inhibitory effect of such hormone as somatostatin released by CCK from the ovine antrum [51]. Some possible mechanisms of CCK action on gastrointestinal motility are also summarized in Figure 6. Similarly to CCK-OP, the inhibitory effect can also be triggered by cerulein confirming the involvement of the same or similar evoking mechanisms [9, 52].

CCK exhibits high affinity to both CCK receptors, CCK-1 and CCK-2 [2]. CCK engages CCK-1 receptor acting centrally on gastric motility in sheep [53]. While in ovine omasum, CCK-1 receptor mediates the action of CCK, in the abomasal antrum, CCK-1 receptor antagonist did not alter the myoelectrical activity [54, 55]. It is thus likely that CCK action in antrum involves CCK-2 receptor and is local. This receptor can be present in antrum since pentagastrin, acting principally via CCK-2 receptor, inhibited antral motility in sheep and also in calves [56, 57, see also 2]. However, it remains unclear whether CCK acted as the gut hormone and/or as neuromodulator.

In the small intestine, Pi used at the lower dose, evoked stimulatory effect, i.e. the premature phase 3, which arrived during short inhibitory period. This finding was also reported in sheep earlier [58]. Since Pi used at the smaller dose evoked the premature phase 3 only in some of the animals studied, it seems likely that its action *via* M_1 cholinergic receptor subtype is not entirely specific comparing with the actions of another selective anticholinergic drug, telenzepine [59-61]. The action of the smaller dose of Pi upon the MMC was stimulatory, but Pi at the higher dose and other anticholinergics inhibited the MMC in sheep and in other species including dog that was reported also by others [26, 27, 62, 63]. Both CCK and cerulein inhibited phase 3 and the whole MMC pattern in sheep and similar effect was observed in other species like dog in response to CCK administration [7, 10, 11,15]. The CCK peptide, applied even at the smallest dose after Pi, converted the premature phase 3 to rebound excitation. Both the mechanism and physiological meaning of this event are unknown. After At, Hx and the higher dose of Pi given alone, the rebound excitation was observed and also described earlier [32, 64]. When CCK peptide administration was followed by the cholinergic blockade, no rebound excitation was observed. Thus, even the small doses of the hormone can prevent undesired (stimulatory) actions of atropine when used, for example, during the intestinal surgery. Since the rebound excitation was regularly evoked during cholinergic blockade, it appears that the non-adrenergic non-cholinergic (NANC) stimulatory neurons underlie its triggering mechanism. In the course of the cholinergic blockade, the vagus-dependent inhibition may be alternated by the stimulation *via* vagal efferent NANC nerves or by other NANC neurons located in the enteric nervous system [65, 66]. Therefore, CCK might be able to exert central, but also peripheral neuronal stimulatory action upon the gastrointestinal tract when the cholinergic receptors are blocked. Stimulatory effect of CCK on duodenal motility in sheep is also consistent with the observations in other ruminant species like calves, in which the CCK receptor antagonist, tarazepide, depressed the duodenal myoelectrical activity [67]. This also concerns the dog, cat and guinea pig [41, 68-70]. It was found in the present study that CCK evokes biphasic or other inconsistent effects upon the ovine small bowel motility what was also observed previously in the rat and sheep [13, 44, 49, 71, 72]. In man, the results are also contradictory. While the *in vivo* study revealed the inhibitory influence of CCK-8 on duodenal motility, administration of CCK antagonist, loxiglumide, decreased the total number of duodenal contractions [30, 43]. In the jejunum, CCK is stimulatory in man, dog and rat [29, 73, 74]. Stimulatory effect could be exerted by direct action of CCK on the small intestinal smooth muscle. It was found that during luminal perfusion of the small bowel by decanoic acid, the CCK-releasing factor, the segmental-type of motor activity was induced [75]. When, during *in vitro* study, CCK was applied, it evoked the ejective pattern [76]. After CCK, jejunal segment ejected fluid bidirectionally, thus the motility pattern evoked by CCK exhibited rather stationary character. After the cholinergic blockade, stimulatory effect of CCK in the small bowel was greatly reduced as compared with the experiments engaging CCK alone, what was observed both in the present and previous studies [11, 13].

The highest dose of CCK peptide, preceded by Hx administration, produced considerably longer spike burst inhibition than Hx given alone. This suggests that the efficient cooperation between CCK and nicotinic cholinergic receptors exists in the gut. Since duration of the spike burst inhibition in the duodeno-jejunum after combined nicotinic-muscarinic blockade followed by CCK administration was the longest, the effects might be additive, at least in part. It has been established that the nicotinic receptors are located in the intramural ganglia of the gastrointestinal tract while the muscarinic receptors are located more distally, mainly on the smooth muscle cells [66]. Therefore, the muscarinic cholinergic blockade inhibited this regulatory pathway although the nicotinic blockade was more effcient since it could block also the non-cholinergic stimulatory neurons. The concept of command (cholinergic) neurons can illustrate this phenomenon further [66].

It was found herein that cholinergic blockade delayed the appearance of phase 3 of the MMC in the small bowel. First phase 3 that arrived afterwards was ectopic and originated from the jejunum. These effects were also earlier described in sheep [63, 64]. The cholinergic blockade is more efficient than vagotomy in the MMC inhibition [22, 77, 78]. CCK is known to exert similar effect, not only in sheep [11, 15]. When CCK was administered after cholinergic blockade, the inhibition of phase 3 of the MMC was prolonged. Therefore, in the small bowel CCK amplified the effect evoked by cholinergic blockade and the question arises whether this effect is additive or synergistic, at least in part. Duration of the inhibitory period was related to the CCK dose, type of cholinergic blockade and region examined. It was reported that CCK and acetylcholine potentiate mutually their effects both in the stomach and in small bowel [3, 79]. When CCK, given under cholinergic blockade inhibited the gastrointestinal motility, especially of phase 3 of the MMC, its action was rather independent of the direct acetylcholine influences. It cannot negate the presence of cooperation between cholinergic and CCK-related mechanisms in the control of gastrointestinal motility, however. In monogastrics, CCK may inhibit contractions in the duodenum acting simultaneously *via* CCK-1 and CCK-2 receptors [18]. It is uncertain whether the same may also occur in sheep. It seems likely that the long inhibition of phase 3 in the duodenal bulb, observed in the present study, occurred because phase 3 in the duodenal bulb of sheep is often absent or reduced. This was also observed previously [80]. The normal (non-ectopic) phase 3 of the MMC originates in ewes most frequently from the duodenum [81]. The duodenal bulb represents the region distinct from the remaining part of the duodenum in sheep [80, 82]. When in sheep, CCK was given alone, it inhibited phase 3 of the MMC for the period shorter than that after the combination of CCK with the anticholinergic drug [63]. First phase 3 of the MMC that arrived following CCK, administered after cholinergic blockade, was also ectopic (it started from the jejunum). Therefore, the CCK-dependent inhibitory mechanisms may cooperate with cholinergic mechanisms, perhaps also during partial cholinergic blockade. Duration of phase 3 inhibition in the jejunum was shorter than that in the duodenum. Most pronounced effects were observed in the jejunum when application of the highest dose of CCK peptide was preceded by nicotinic or nicotinic-muscarinic blockade. Thus the effect of CCK upon the MMC appears to be evoked principally *via* CCK receptors located within the enteric nervous system (both CCK 1 and CCK 2 receptors, see [2]), possibly in the cooperation with other (maybe central) neurons. The direct action of CCK on the small intestinal smooth muscle also cannot be excluded although it appears more feasible in the control of the spike bursts than in the control of the MMC. CCK can be released from I cells located in the duodenum and jejunum [1]. In ruminants, presence of I cells in the small bowel is questionable although CCK can be released from this region as CCK-OP [83]. During the cholinergic blockade, circulating CCK was probably unable to act *via* the central nervous system. It was reported that CCK is not able to cross the blood-brain barrier [25] thus it seems likely that peripheral CCK cannot act centrally. Whether this is really true or not in various animal species is not known since it was demonstrated in rats that peripherally administered CCK acted on the brain stem neurons [84]. However, it has been recognized that CCK, most probably released from the peripheral neurons and/or from the I cells, can evoke the discharge of

vagal neurons acting through CCK-2 receptors located on vagal afferents, while both CCK receptors are present in vagus nerve [85]. Stimulation of vagal afferents enhances neuronal transmission in the nucleus of the solitary tract, activates central CCK-1 receptor pathway and possibly also acts in other centers of the brain. These actions may disrupt the MMC [86]. It was also reported that capsaicin affected CCK action on gastrointestinal motility in rats that confirms further that this mechanism exists [72, 87]. Therefore, peripheral CCK may act centrally omitting the blood-brain barrier at least in some species (see Figure 6).

The inhibition of the MR in the duodenal bulb was longer than in the duodenum suggesting that the latter region represents the main site of MR initiation. Although the MR undergoes cholinergic influences what was found in this and previous studies [23, 28], almost nothing is known as to the localization and contribution of the cholinergic receptor subtypes involved in the control of the pattern. At given intracerebroventricularly in rats remained without effect upon the MR evoked centrally by naloxone [88]. Thus, the character of central control of the MR remains unclear. When CCK peptide injection followed the nicotinic blockade, the highest dose of CCK-OP was the most effective in the MR inhibition observed in the duodeno-jejunum. Cerulein often exerted more pronounced effect in the jejunum than in the duodenum. These differences between the effects evoked by both CCK peptides suggest that the mechanism of action of these CCK peptides in the gut may be similar, but not be the same. Presented results indicate that CCK, exerting its action under cholinergic blockade, is able to inhibit the MR appearance while the nicotinic blockade is more efficient than the muscarinic blockade (see Figure 7). It seems likely that the mechanisms controlling the MR in the small bowel can be similar to those controlling the arrival of the spontaneous spike bursts.

Both lower doses of CCK-OP used in the study were physiological. The highest dose also appeared to remain within the physiological range, perhaps at its upper border [39]. When CCK exerts the inhibitory action on the gastrointestinal motility *via* neuronal pathway, the greater dose of exogenous hormone may be required. Therefore, the highest dose of CCK could be treated as the physiological one. This may also depend upon the site of CCK action. When CCK acts as a gut hormone its physiological dose can be greater than when it acts as a neuromodulator. In sheep it is an unexplored question while it appears that both these pathways can be taken into account. Cerulein doses used in this study, i.e. 20 times lower than that of CCK-OP, appeared to be relevant to the doses of CCK-OP, although it was suggested that cerulein is only 8-15 times times stronger than CCK-OP [see 14]. The long inhibition of phase 3 of the MMC by combined actions of both the anticholinergic drugs and CCK may result also from the cooperation with other regulators like gastrin and somatostatin acting centrally or peripherally [19, 89, 90]. Both these hormones inhibit the arrival of phase 3 of the MMC [56]. The release of somatostatin from the upper gastrointestinal segments is possible in this situation, since it may be independent of the cholinergic system. This view is based upon the observation of Bell et al. [4] that in the calf somatostatin secretion was not blocked by vagotomy. Furthermore, the cooperation of CCK with other inhibitory regulators as opioids and with some other, like secretin, glucagon, VIP and GIP cannot be excluded [79, 91].

5. CONCLUSIONS

It is concluded that in sheep:

1) cholinergic system modulates CCK action upon the gastro-intestinal motility,
2) inhibitory actions of CCK upon the gastro-intestinal motility, observed after cholinergic blockade, were dose-dependent,
3) CCK, acting under cholinergic blockade, prevents the arrival of normal and premature phase 3, ‘minute rhythm and rebound excitation in the gut,
4) cooperation between the cholinergic system and CCK, regarding the inhibition of the gastrointestinal motility, is most efficient when the nicotinic receptors are involved,
5) following the application of cholinergic blockade the effects of cerulein upon the gastrointestinal motility were comparable with those of CCK-OP,
6) mechanism of pirenzepine action on gastro-intestinal motility is dose-related,

7) the question whether CCK acts as a hormone or as neuromodulator still remains unclear.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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